Study of the role of *Sirtuin1* (*dsir2*) gene in Alzheimer's disease model of *Drosophila melanogaster*

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Introduction

Chapter 1

Sirt1 modulate the AD related pathologies shown by Drosophila melanogaster model of AD

1.1 Introduction

As mentioned in the literature review, Sirtuins are known as NAD+ dependent class III histone deacetylases enzyme play a key role to control cellular functions i.e. metabolism and ageing. Previous studies have shown that Sirtuins has a protective role against cardiovascular disease, cancer and other age-related neurodegenerative disease (NDDs), etc. Moreover, Sirt1 has been identified in various neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases. Sirt1 might be a key target for designing the novel therapeutic target for AD.

Chapter 2

Improvement in AD-related pathologies was associated with reduction in cell death due to overexpression of Sirtuin1 in AD genetic background.

2.1 Introduction

As mentioned in chapter 1 overexpression / downregulation of Sirt1, modulate the AD-related pathologies i.e. rough eye phenotype, behavioral defects (climbing and phototaxis), reduced body weight, reduced survival in AD model flies etc. Since, neuronal cell death is one of the key pathologies associated with AD and several studies have demonstrated that excessive cell death in Drosophila is mainly associated with increased expression of pro-apoptotic genes (Grim, Reaper and head involution defective {Hid also known as Wrinkled}) and altered expression of Drosophila Inhibitor of Apoptosis Protein 1 (DIAP1) results in cell death in AD model (Goyal et al. 2000; Tare et al. 2011; Wing et al. 2002). Thus, the improvement seen in AD pathologies in Sirtuin1 overexpression genetic background might be due the rescue in neuronal cell death. Thus, herein, we aimed to explore the status of cell death and expression of cell death genes in AD model flies alone and in Sirtuin 1 overexpression /downregulation genetic background. We have presented a brief description of cell death its participation in AD and apoptosis in Drosophila.

Chapter 3

Sirtuin 1 gene interacts with JNK and Notch signaling in Drosophila

3.1 Introduction

As shown in chapter 2, AD model flies showed excessive cell death in eye and brain cells. Further, overexpression/downregulation of Sirt1 modulate the excessive cell death shown by AD model flies (GMR-GAL4-UAS-TauWT/+;++; GMR-Aβ42k52/+; GMR-Aβ42k53/+) by reducing the expression of pro-apoptotic genes grim, reaper, hid and increasing the expression of diap1 (Drosophila inhibitor of apoptosis). As mentioned in chapter 1, the accumulation of amyloid beta 42 and Tau tangles are associated with the onset of Alzheimer's diseases. The extracellular/intracellular accumulation of these proteins might result in generation of cellular stress thus, activation of stress signaling such as JNK (C-Jun N-terminal kinase) and ultimately cell death as shown in chapter 2. Also, Notch signaling plays a crucial role during development, thus, in this chapter, I have examined the participation of JNK and Notch signaling in AD pathologies and its possible interaction between Sirt1 and AD-associated genes in Drosophila.

Summary

4. Summary

In summary, the present study suggest that overexpression of *Sirt1* improves the AD-related pathologies in *Drosophila* by rescue in rough eye phenotype, improving the climbing and phototaxis activity along with reduced cell death in neuronal tissue, reduced expression of apoptotic gene (*Grim, Reaper, Hid*), reduced $A\beta_{42}$, *Appl* and *Tau* expression and reduced *JNK* and *Notch* expression. On the other hand, downregulation of *Sirt1* enhanced the rough eye phenotype, increased climbing and phototaxis defect along with incrased rate of apoptosis, incrased apoptotic gene (*Grim, Reaper, Hid*) expression, increased expression of AD genes (*Aβ, Appl* and *Tau*) and *JNK* and *Notch* signaling in AD model flies.

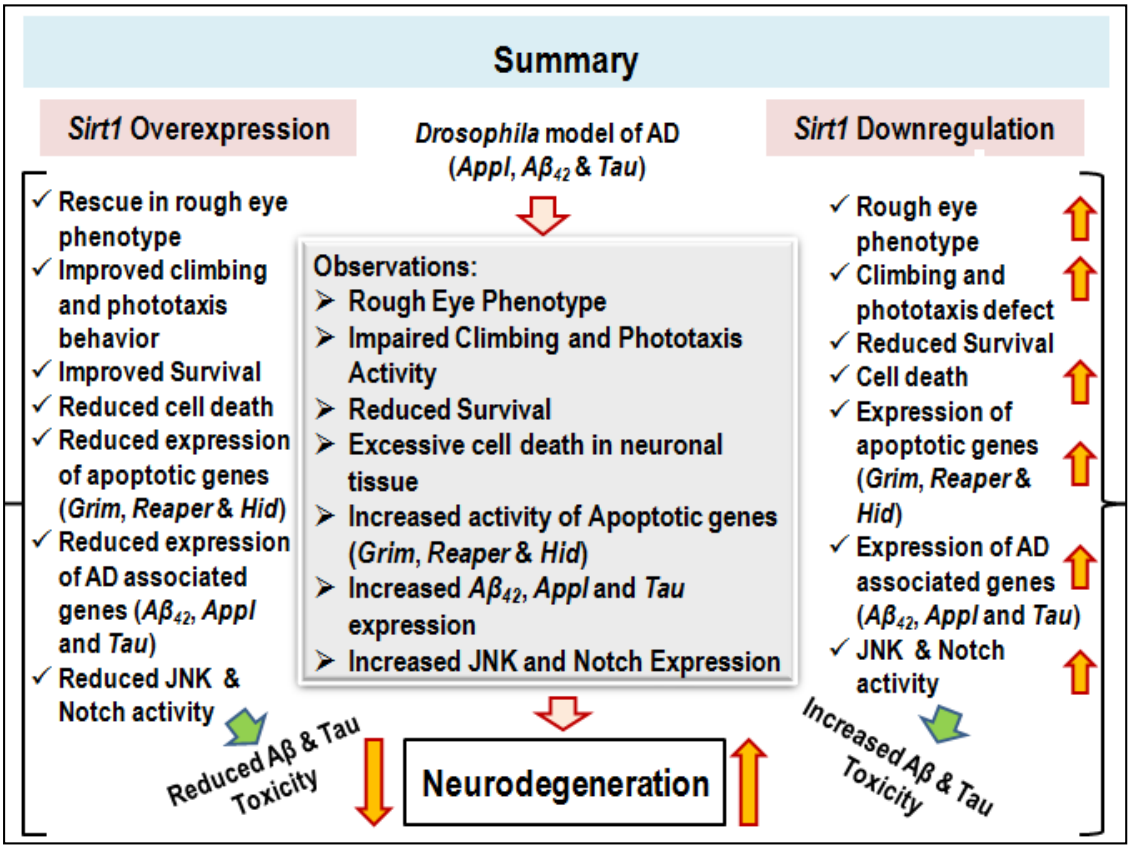
Summary
Sirt1 Overexpression
Drosophila model of AD
(Appl, Aβ42 & Tau)
Sirt1 Downregulation
✓ Rescue in rough eye phenotype
✓ Improved climbing and phototaxis behavior
✓ Improved Survival
✓ Reduced cell death
✓ Reduced expression of apoptotic genes (Grim, Reaper & Hid)
✓ Reduced expression of AD associated genes (Aβ42, Appl and Tau)
✓ Reduced JNK & Notch activity
Observations:
➢ Rough Eye Phenotype
➢ Impaired Climbing and Phototaxis Activity
➢ Reduced Survival
➢ Excessive cell death in neuronal tissue
➢ Increased activity of Apoptotic genes (Grim, Reaper & Hid)
➢ Increased Aβ42, Appl and Tau expression
➢ Increased JNK and Notch Expression
✓ Rough eye phenotype
✓ Climbing and phototaxis defect
✓ Reduced Survival
✓ Cell death
✓ Expression of apoptotic genes (Grim, Reaper & Hid)
✓ Expression of AD associated genes (Aβ42, Appl and Tau)
✓ JNK & Notch activity
Reduced Aβ & Tau Toxicity
Neurodegeneration
Increased Aβ & Tau Toxicity

Figure S1 : Summary figure showing the effect of overexpression/ downregulation of *Sirt1* in the modulation of AD-related pathologies in *Drosophila*

Conclusions

5. Conclusions

On the basis of above results the following conclusions were drawn

Chapter 1

- Overexpression/downregulation *Sirt1* modulate the rough eye phenotype, behavior abnormalities, reduced body weight, and reduced survival in AD model flies.
- Overexpression/downregulation of *Sirt1* modulate AD associated genes (*$A\beta_{42}$, Tau* & *Appl*) expression in AD model flies.
- Overexpression/downregulation of *Sirt1* modulate *Sirt1* expression in AD model flies.

Chapter 2

- Overexpression/downregulation of *Sirt1* modulate the cell death in third instar larval eye imaginal disc and larval brain of AD model flies.
- Overexpression/downregulation of *Sirt1* modulate pro-apoptotic genes (*Grim, Reaper* and *Hid*) expression in AD model flies.
- Overexpression/downregulation of *Sirt1* modulate the *DIAP1* expression in AD model flies.
- Overexpression/downregulation of *Sirt1* modulate $A\beta_{42}$ expression in AD model flies.
- Overexpression of *Sirt1* along with overexpression of *p35* rescue the rough eye phenotype in AD model flies.

Chapter 3

- Overexpression of *Sirt1* along with downregulation of *JNK* rescue rough eye phenotype and increased the behavior activities in AD model flies.
- Overexpression of *Sirt1* along with downregulation of *JNK* reduced pro-apoptotic genes (*Grim, Reaper* and *Hid*) expression increased expression of *DIAP1* in AD model flies.
- Overexpression/downregulation of *Bsk* modulated *Sirt1* expression in AD model flies.

- Overexpression of *Sirt1* along with downregulation of *Notch* rescue rough eye phenotype and increased the behavior activities in AD model flies.
- Overexpression of *Sirt1* along with downregulation of *Notch* reduced pro-apoptotic genes (*Grim, Reaper* and *Hid*) expression and increased expression of *DIAP1* in AD model flies.
- Downregulation of *Notch* reduced *Delta* expression in AD model flies.
- Overexpression/downregulation of *Sirt1* modulate *JNK* and *Notch* expression in AD model flies.
- Overexpression/downregulation of *Sirt1* modulate p-JNK , NICD and Delta expression in AD model flies.